IMPACT OF TURBULENCE AND GROWTH RATE ON THE SCATTERING SIGNATURES OF MARINE PHYTOPLANKTON

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LONG-TERM GOALS

The long-range goal of my ONR-sponsored research has been to use bio-optical techniques to understand the distributions of phytoplankton, in space and time in the sea. An important control in this effort is to examine physical factors which affect particle size and shape (which would change their optical volume scattering function). Turbulent shear is one such process which can have profound effects on the shape of large cells, and the length of filamentous chains and is the focus of my work in year 1. In year 2, I will focus on a method for identifying particles in the sea using their depolarization scattering properties. Phytoplankton species contain unique arrays of organelles and subcellular particles which depolarize light to varying degrees. I am using the angular dependence of depolarization to aid in identification of phytoplankton species (and other particles).

OBJECTIVES

The objectives of this work are to:
Year 1
- Examine the role of micro-scale turbulence in the rate of aggregate formation in filtered sea water, and its subsequent impact on the bulk volume scattering function.

-Examine the role of micro-scale turbulence in affecting the volume scattering functions of a range of phytoplankton species. Turbulence will be varied from those typical of quiescent mid-water environments, highly tidally-mixed environments and extremely high turbulence levels found in a flow-through fluorometer or beam transmissometer.

Year 2
-Measure the volume scattering function of various phytoplankton species either with the same vertical polarization as the incident laser beam, or after the plane has been rotated 90^{o} to the horizontal. The objective is to verify the conservative nature of the ratio of these two volume scattering functions (β_h/β_v) versus solid angle within an algal taxa (such that they could be used for identification along with other traditional optical indicators such as fluorescence and/or absorbance).

APPROACH

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Turbulence generation

To generate quantified micro-scale turbulence, an oscillating grid approach has been used, in which a grid is reciprocated through a cylindrical optical cuvette at a precise rate using a computer-driven stepping motor. Calculation of the energy dissipation rate (ε; Watts kg^{-1}) imparted to the sample requires measurement of the stress to push the grid through the water (S= newtons m^{-2}; measured using a precision balance and knowing the area of the bottom of the cuvette through which the stress is conveyed), the volume of fluid which passes through the grid (V_{grid}; m^{-3}) and T_{stroke}, the time for the grid to move from the top of the stroke to the bottom (1/2 of a complete cycle). We must also correct for any Archimedes effect from the grid shaft which moves in and out of the fluid. Energy dissipation is calculated as:

$$\varepsilon = [(S \times V_{grid} / T_{stroke}] / (V_{cuvette} \times \rho)$$

where $V_{cuvette}$ is the cuvette volume ($V_{cuvette}$; m^3) and ρ is density (kg m^{-3}). When all of the fluid moves by the grid in one stroke, then the above equation simplifies to:

$$\varepsilon = (S \times \rho) / T_{stroke}$$

With knowledge of the kinematic viscosity (γ; $m^2\ s^{-1}$), the equivalent turbulent shear (G; s^{-1}) is calculated as:

$$G = (\varepsilon / \gamma)^{1/2}$$

Finally, the Kolmogorov eddy length scale (η ; m) is calculated as:

$$\eta = (\gamma^3/\varepsilon)^{1/4}$$

Given quantitative energy dissipation, volume scattering of the samples are monitored using a laser light scattering photometer. The approach to the control experiments is to quantify any changes in volume scattering as filter-sterilized seawater is examined in a sterile cuvette. For the experimental samples, cultures are carefully introduced into the cuvette, and short-term (several minutes) measurements are made at a given energy dissipation level. The process is repeated several times, with and without turbulence to verify the values. Microscopic enumeration is used to estimate cell concentration and chain length.

WORK COMPLETED

The first major task concerned the experimental set-up. The laser light scattering photometer was converted from a He-Ne laser to an Argon ion laser (to work with wavelengths of light more representative of the sea). The original proposal was to generate micro-scale turbulence using standard pipe flow, in which large volumes of algae were run through a pipe (through the center of the light scattering photometer), with a

screen placed upstream of the interrogation volume. Thermal anemometry was to be used to quantify the micro-scale turbulence. A subsequent problem with this design was that it was difficult to maintain sterile conditions with such large volumes, and the pumping of cells meant that they would be exposed to much higher shear rates than we were generating in the pipe flow. With an oscillating grid, micro-scale turbulence could be much more easily achieved within a sterile cuvette sitting directly within the light-scattering photometer, with smaller volumes of culture required. Moreover, the combination of using a stepping motor to power the grid (~6000 steps per 3 cm) combined with a precision balance to measure the grid stress, provided the capability of making very fine-scale measurements of energy input through the grid. The major hurdle of the design was to make sure that boundary effects around the grid were minimized, and to construct the grid with crossing bars instead of a plate with holes. With the latter, there would be a higher solidity ratio (solid area:open area), which gives greater momentum to the flow, causing potential mean recirculating flow pattern, not turbulent flow. Given the potential limitations of all of the designs, the goal was to produce relative differences in energy dissipation using differing grid oscillation speeds, realizing that absolute dissipation rates may be difficult to calculate at the small scale of our cuvette.

Control Experiments

Control experiments involved adding 0.2μm pore-size filtered seawater to a pre-sterilized, ultra-clean cuvette. This water was continuously circulated through a 0.2μm pore-size filter cartridge while the cuvette was sitting in a Wyatt Dawn laser light scattering photometer. When the volume scattering function and backscattering values stabilized at values seen in pure seawater, filtration was stopped, and the volume scattering function was monitored over time either with no turbulence imparted to the seawater, or with the grid oscillating. As aggregates increased in size, the change in volume scattering was measured.

Algal measurements

Species of phytoplankton were grown in batch culture in K media (with Si added for diatoms). They were carefully transferred to the optical cuvette, and measurements first were made on the stability of their volume scattering function in quiescent water. Then energy was imparted by oscillating the grid and again watching the volume scattering function. Care was taken to minimize sheer when sampling cells for microscopy.

RESULTS

There have been several developments over the course of the first phase of this research. Our control experiments have demonstrated the importance of submicron aggregation/polymerization to particle scattering. Prior to these experiments, we had traditionally observed that 0.2μm filtered seawater left in a closed container would increase its backscattering over time. Recent results of this project demonstrate that this process is biologically-mediated (autoclaved water will not form sub-micron aggregates). Rather, filter sterilized seawater will continually increase its particulate backscattering by as much as a factor of 2-3X. If this water is filtered again, the process will repeat itself. But note, cells do not pass the filtration process, so the aggregation likely represents the

production of biologically active, sub-micron particles . As part of these experiments, we have established the time constants of the process.

In terms of the species volume scattering functions, relatively minor changes were observed as the equivalent turbulent shear was increased from quiescent conditions to ~2 s^{-1}. Such results even apply for large diatoms such as *Ditylum brightwellii*, all the way down to small diatoms such as *Skeletonema costatum*. Thus, the null hypothesis is accepted, that the volume scattering function appears to be relatively stable over a range of turbulence levels. As of this writing, the remaining species measurements are being completed. There is a complicating effect of growth phase on these measurements, but it is more related to cell sinking, than changes in the volume scattering function. It has long been known that cells sink faster when they are in stationary phase than when they are in logarithmic growth. Thus, at lower turbulence levels, the cells cannot be maintained in suspension, thus they sink below the laser beam, giving the *appearance* of lower cell-specific volume scattering. This was easily controlled, however, by keeping measurements of short duration.

IMPACT/APPLICATIONS

The first impact of these results is based on the control experiments in which variability on the order of 2-3X was observed in the backscattering of 0.2µm-filter-sterilized seawater. This variability likely represents the aggregation of biologically-active, sub-micron particles. Since dissolved and particulate scattering are usually calculated by difference between an unfiltered and 0.2µm-filtered samples, then if the scattering of dissolved blanks vary by 2-3X, this will translate to associated differences in particle scattering. These results also have consequence to understanding reflectance in the sea because reflectance is a function of absorption and backscattering (Gordon et al., 1988). Higher backscattering attributed to a dissolved "blank" will mean that the backscattering (and reflectance) attributed to particulate material will be similarly reduced.

The other impact of this work is that if we can assume that volume scattering functions of particulate matter are stable under variable turbulence in the sea, then scattering changes can be attributed to processes of particle production, destruction, advection, upwelling and sinking, not *in situ* changes associated with transient conditions of micro-scale turbulence. That is, turbulence measurements over short time scales apparently are not required to interpret optical data. The secondary factor of cell sinking is obviously closely tied to turbulence via particle suspension, however, which can obviously affect the inherent optical properties of the water column. The investigation has turned to understanding the implications of well-known cell size vs. sinking relationships coupled to cell size vs. scattering relationships, to understand how this would affect the dynamics of optical properties of the upper ocean after a mixing event.

TRANSITIONS

The project is now in the last phase of the turbulence experiments, and experiments on the polarization properties of various phytoplankton species are about to begin.

RELATED PROJECTS

Collaborative relationships are maintained with Dr. Ken Voss and Dr. Howard Gordon, both ONR-funded investigators at the University of Miami Dept. of Physics. Discussions with Dr. Alan Brandt (Applied Physics Laboratory, Johns Hopkins University) were very helpful in the experimental design as well as the interpretation of the oscillating grid results.

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